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REVIEW ARTICLE

Mechanism Behind and Future Prospective of CRISPR/Cas9 as Revolutionizing Genome Editing Technology

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Abstract

The recent genome editing tools, such as Transcription Activator Like Effector Nucleases (TALEN), Zinc Finger Nucleases (ZFN) and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) have been adapted rapidly to manipulate genes. CRISPR associated cas9 provides unparalleled control over gene editing. The CRISPR/Cas9 system works in a sequence-specific manner as it recognize and cleave foreign DNA or RNA. The first experimental evidence of CRISPR Cas9 was provided by experiments of *Streptococcus thermophiles* (Lactic Acid Bacterium). For functional genomic studies, CRISPR screens provides a practical way. As suggested by existing evidence CRISPR screen is more specific and more reliable as compared to RNAi screen. CRISPR Cas9 has also used for intracellular defense against foreign DNA. In medical genetic researches the versatility, reliability and specificity of CRISPR is proved to be a promising player. In this review we tried to summarize the potential applications of this system in various research domains.

Keywords: Genome editing, RNAi, CRISPR, cas9, ZFN, TALEN

Introduction

In biology, over the past 60 years technologies for manipulating DNA have enabled us for many of the advancements. Over the past two decades the advent of genome sequencing technologies for large and different types of organisms has been one of the singular advancement. Since the discovery of DNA double helix many researchers has been cogitate on the possibility of site specific changes into the genome of different organisms. To manipulate gene in a variety of animal and cellular models, the recent genome editing tools, such as Transcription Activator Like Effector Nucleases, Zinc Finger Nucleases and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) have been adapted rapidly to manipulate genes (Chadwick et al, 2017). CRISPR associated cas9 is the most powerful category of gene editing which provides unparalleled.

Control over gene editing. CRISPR cas9 can be adapted to different targets as it is flexible and easy to use. CRISPR loci and Cas proteins are evolved as a defense against viruses in 50% of bacteria (Khalili et al, 2017). CRISPR (Clustered regularly inter-spaced short palindromic repeats) was described as a series of short direct repeats inter-spaced by short sequences in *Escherichia coli* genome (Ishino et al, 1987). In numerous bacteria and archea CRISPR was later detected, and certain predictions were also made about their role in gene regulation or DNA repair. Cas9 is an endonuclease

enzyme that functions in Prokaryotes Adaptive Immunity (Mojica et al, 2000). But these genome editing tools are similar in the way that they consist of two domains, a catalytic domain to make double stranded breaks (DSBs) and the programmable domain for recognition of specific DNA sequences.

CRISPR/ Cas9 system in bacteria work as an adaptive immune system which protects bacteria against invading foreign DNA, such as that of a bacteriophage. This system consists of CRISPR loci in the genome and a Cas9 protein (Makarova et al, 2002).

Mechanism of CRISPR/Cas9

CRISPR is a tandem direct repeat sequences at a specific locus in genome and Protospacer (the spaces in between repeat sequences) both of which are derived from the invading elements such as phages (Brookhouser et al, 2017). The story of CRISPR started in 1987 by the study of *iap* enzyme which involved in the conversion of alkaline phosphatase in *E. coli.* Scientist reported that unusual set of 29bp repeats were found at the downstream position of the *iap* gene. Later find out that these N terminus repeats were interspaced by intervening non-repetitive sequences. Over the next 10 years by the advancement in the field of genome sequencing as the time passes, more microbial genome were sequenced. Some other additional repeat elements were also reported in the genome of bacterial and archaeal strains. This Cas genes further serve as a bases for the classification of CRISPR in to three different types (Ishino et al, 1987; Kim et al, 2014). The type I and type III CRISPR loci contain multiple Cas proteins, these Cas proteins form complexes with crRNA for the recognition and destruction of target nucleic acids (Haft et al, 2005; Makarova et al, 2015). But in the type II system there is significantly reduced number of Cas proteins. However, their biological significance remained elusive.

In 2007 the first experimental evidence of CRISPR Cas was provided by experiments of *Streptococcus thermophiles* (lactic acid bacterium). This was an exciting discovery as previous studies showed that the CRISPR loci could transcribed (Brouns et al, 2008), and that phage are unable to infect archaeal cells carrying spacers corresponding to their own genomes (Graveley et al, 2009). All these findings led to the speculation that CRISPR serve as a defense mechanism in bacterial and archaeal system, and individual spacer sequence facilitate the bacteria to protect from the phage reinfection (Pourcel et al, 2005). For biotechnological purpose the first successful application of CRISPR is to utilize CRISPR/Cas system present in cultured bacteria used in dairy industry for immunization against phages (Barrangou et al, 2007).

The CRISPR/Cas9 system works in a sequence-specific manner as it recognize and cleave foreign DNA or RNA. The CRISPR mechanism can be divided into three stages: (i) adaptation or spacer acquisition, (ii) crRNA biogenesis, and (iii) target interference. In a first phase, a distinct sequence of the invading Protospacer is incorporated into the CRISPR system forms a new spacer. The incorporation of Protospacer into the CRISPR system creates memory in the host organism by memorizing the invading genetic material and display the adaptive nature of this immune system (Wright et al, 2016). Cas1 and Cas2 are two proteins which are ubiquitously involved in the spacer acquisition process because it is found in all CRISPR/Cas types except type III and IV CRISPR/Cas systems because they have no homologous Cas proteins (Shmakov et al, 2015).

In past, there is an advancement in revealing the genetic and biochemical principles of CRISPR Cas9 immunity but the mechanism through which spacer acquisition, the selection of spacer and it's integration is not fully understood (Fineran et al, 2012). By the advancement in the field of molecular biology and biochemistry it has been found that Cas1 and Cas2 of the type I-E system forms a complex that complex promotes the new spacers integration in a manner that is similar of viral Integrases and transposases. As it has been known that both Cas1 and Cas2 proteins are nucleases, for spacer acquisition the catalytically active site of Cas2 is dispensable. A new spacer incorporate at the leader-repeat sequence of the CRISPR system while the first repeat of the CRISPR system is duplicated (Marraffini and Sontheimer, 2008). The selection of integration of target sequence into the CRISPR locus is not arbitrary. A short sequence, called the Protospacer Adjacent Motif (PAM) in type I, II and V CRISPR/Cas systems is located directly next to the Protospacer and is essential for integration and inter-

ference (Hale et al, 2009). This PAM recognizing domain of Cas9 is responsible for selection of Protospacer. The frequency of integrated spacers increases in the presence of the interference complex that are adjacent to PAM motif (Barrangou et al, 2007). Through priming, the increased uptake of new spacers can be stimulated. This process displays a distinct adaptation mode compared to naive spacer acquisition as it strictly requires a pre-existing spacer matching the target. The Protospacer which are present in close proximity to target site have high acquisition rate (Gilbert et al, 2014).

Future Perspective

The landscape of biological research has transformed by numerous powerful tools originating from bacteria and phages. Modern genetics may be revolutionized by CRISPR/Cas9 in many ways i.e. comp-arable to the advent of recombinant DNA technologies. For functional genomic studies CRISPR screens provides a practical way. Multiple screen formats can be carried out with CRISPR cas9 as CRISPR screen is more versatile as compared to conventional screens conducted with cDNA or RNAi libraries. As suggested by existing evidence CRISPR screen is more specific and more reliable as compared to RNAi screen. In medical genetic researches the versatility, reliability and specificity of CRISPR is proved to be a promising player. In various functional genomics CRISPR screens have been applied such as to identify genes that are essential for cell survival, genes that are promoting cancer cell metastasis and genes that are involved in resistance to drugs and toxins. As a novel anti-virus tool CRISPR/cas9 can be adapted into human cells (Cong et al, 2013). According to a study published, alteration in gene expression have been restored through introduction of deletion mutations during tumorigenesis in somatic cells and germ cells, by the use of advanced CRISPR/cas technology (Aqeel and Raza, 2017). Type II CRISPR-SpCas9 protein from *Streptococcus pyogenes* have applied successfully for targeted genome editing in diverse organisms and cell types by several research groups.

In some studies CRISPR/Cas9 has also used for intracellular defense against foreign DNA. It was found that CRISPR/cas9 can disrupt latently integrated viral genome and it also provides adaptive defense against new virus infection, replication and expression in human cells. CRISPR applications are just starting to emerge (Kaminski et al, 2016), It is already used by clinical researchers to create treatment for cancer i.e. tissue based and it can also be used for the treatment of other genetic disorders (Travis and John, 2015).

Conclusion

In short, if scientists can dream of a genetic manipulation, CRISPR can make it happen now. During the human gene-editing summit, Carpenter at one point described its capabilities as "mind-blowing." It's the simple truth that for better or worse, we are now living in CRISPR's world.

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